Inhibition of granulocyte-macrophage (GM) colony formation by basic polypeptides from mouse Ehrlich ascites tumor cells

M. Szmitkowski, H. Gabryel and R. Farbiszewski

Department of Clinical Diagnostics, Institute of Biochemistry and Medical Analytics, and Department of Inorganic Chemistry, Institute of Chemistry and Biophysics, Medical School, 15-276 Białystok (Poland), 2 March 1981

Summary. The polypeptides isolated from mouse Ehrlich ascites tumor cells were tested for their inhibitory activity against GM colony formation. It was found that basic polypeptides with low molecular weight evidently inhibit colony formation. Our data reveal that the tested polypeptides may show chalone activity.

The in vitro proliferation and differentiation of cells is controlled by the presence of family glycoproteins termed colony stimulating factors (CSF). Many data have been presented which show that CSF function is intimately linked with the regulation of granulopoiesis. On the other hand, the growth of granulocytes and differentiation can be inhibited by several substances such as serum lipoprotein (inhibitor of CSF)[1], lactoferrin (inhibitor of CSF biosynthesis)[2] and specific chalones[3].

Production of chalones takes place in mature granulocytes. Chalones have been precisely characterized by Paukovits et al.[3] as polypeptides with mol.wts ranging from 5000 to 10,000. Recently it has been demonstrated that ninhydrin-positive substances with a mol.wt of approximately 7000–20,000 daltons are produced and secreted by human sarcoma and other carcinoma cells[4] different tumor cell lines in culture[5,6] and chemically transformed cells[7].

Therefore, our attention has been directed to the study of the effect of glycopeptides and polypeptides isolated from Ehrlich ascites tumor cells on the growth of granulocytes and macrophages in culture.

Materials and methods. Cytosol ^{3}H-arginine containing polypeptides was prepared as follows. The tumor-bearing mice were fasted 18–20 h before i.p. injection of 0.2 ml 20 µCi L-5-^{3}H arginine, sp. act. 16.7 Ci/mM, Radiochemical Centre, Amersham, England. 30 mice were killed 2.5 h after injection of arginine. The further procedure for cytosol preparation was the same as described previously[8].

The cytosol was submitted to gel filtration on Sephadex G-25 in order to separate the high molecular-weight from the low molecular-weight substances. Peak II, containing the substances of low mol.wt, was pooled and desalted on the column with Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, California). Fractionation of the polypeptide mixture was carried out by chromatography on SP-Sephadex C-25. Full details are given elsewhere[9]. Homogeneity of these polypeptides was confirmed by isoelectric focusing, polyacrylamide disc electrophoresis and high voltage paper electrophoresis.

1 glycopeptide and 4 polypeptides were used throughout the experiments. Nos IV and V were basic in character and No. V was rich in arginine.

Colony (> 50 cells/clone) formation was determined by plating 10^5 mouse bone marrow cells in 1 ml of 0.3% agar medium, containing 0.05 ml of particular polypeptides (100 µg/ml) and 0.05 ml of stimulant (1:2 diluted serum of mouse treated with *Escherichia coli* endotoxin as an agent enhancing CSF level). Cultures were incubated at 37 °C in a humidified atmosphere containing 7.5% CO_2 in air for 1 week. Colonies and clusters were scored from 3–5 plates for each assay point. As a control 0.9% NaCl was used instead of the tested polypeptides.

Results and discussion. Polypeptide No. I, with a high mol. wt, and the next 2 polypeptides (Nos II and III) have no effect on GM colony formation. Basic polypeptide No. IV (mol.wt 10,000, pI 8.7) and polypeptide No. V (mol.wt 8500, pI 8.9), which is rich in arginine, are evidently potent inhibitors of GM colony formation (table).

The mechanism of this inhibition may be interpreted on the basis of 2 hypotheses; a) the polypeptides may act as inhibitors of CSF; b) the polypeptides possess inhibiting activity as a chalone. The experimental system used precludes the inhibiting action of ascites cell peptides at the level of CSF biosynthesis. A direct inhibitory effect of peptides IV and V on added CSF is, however, not excluded. In our opinion these polypeptides can act as a chalone with low mol.wt, as described by Paukovits et al.[3].

It has recently been reported that cancer cells inhibit erythropoiesis[10] and that a higher level of serum inhibitor activity on GM colony formation is found in cancer patients as well[10]. Furthermore, it has also been reported by Nimberg et al. that a polypeptide from the serum of cancer patients has the ability to suppress lymphocyte blastogenesis in mice[12]. These results suggest that polypeptides secreted by tumor cells could be specific inhibiting factors (chalones) of granulopoiesis.

Finally it should be noted that the experiments were carried out in mice, and polypeptides were isolated from a controlled pure Ehrlich ascites line, which was freed from granulocytes.

The effect of polypeptides from Ehrlich ascites tumor cells on GM colony formation

Material No.	Sample	Mol.wt	pI	No. of colonies	Inhibition (%)
0	0.9% NaCl	–	–	60±5	–
I	Glycopeptide	18,500	5.0	60±7	0
II	Peptide	11,000	7.7	58±6	3
III	Peptide	10,500	7.7	59±6	2
IV	Peptide	10,000	8.7	42±4	30
V	Peptide	8,500	8.9	40±4	33

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